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To: . Art Palmer
From: . S. Tafur, D. Faustini and J. Charles
Subject: . Patent Disclosure Supplement for D-114-Cigarette Beetle Control

Date: August 1, 1983

A recent experiment was completed^{1,2} in the continuing study of the toxic principle(s) of cigarette beetle pupal cell (PC) casing. The purpose of the test was to develop a faster and simpler means of feeding bioassay. This was necessary since the methanol extract of PC casing, which has previously been shown to be toxic,³ is hygroscopic and difficult to handle. The preceding toxicity results (45% mortality at 15% w/w on bright tobacco) were somewhat tenuous since the accurate observation of larvae was obscured by the sticky, globular nature of the feeding matrix.

The design of this experiment was based on the premise that CB larvae could survive without any feeding material for several weeks. Exposure to varying doses of test sample was expected to demonstrate toxicity either by contact with the sample or by ingestion. Fructose-containing samples provided a means of testing the larvae with an adequate nutrient source in case their survival without food was not long enough to observe toxicity due to the test samples.

In the assay the cigarette beetle (CB) larvae were placed in vials either with no nutrient supply (i.e. empty vials) or with 5 mg fructose. The test samples consisted of PC methanol extract⁴ at 2.0 mg/vial or 0.5 mg/vial with or without fructose. A positive control sample of malathion (a known contact pesticide) was also included.

The difficulty in handling the PC methanol extract was overcome by preparing a solution of extract in water (20 mg/ml) and then dividing into appropriate aliquots for testing. The volumes were kept minimal (2.0 mg = 100 μ l and 0.5 mg = 25 μ l) and samples were dried under nitrogen. The 5 mg fructose aliquots were individually weighed into vials, dissolved in 50 μ l water, and dried under nitrogen. For the test samples containing the PC methanol extract mixed with fructose, the volume of water from the solution of methanol extract was sufficient to ensure uniform mixing prior to drying under nitrogen. A list of controls and test samples is in Table I.

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TABLE I. SAMPLES SUBMITTED FOR CIGARETTE BEETLE FEEDING BIOASSAY

<u>Sample Code^a</u>	<u>Sample Description</u>
I	Control (no food source)
II	Pesticide Control (malathion)
III	PC Methanol Extract (2 mg/vial)
IV	Fructose Control (5 mg/vial)
V	Fructose (5 mg/vial) + PC Methanol Extract (0.5 mg/vial)
VI	PC Methanol Extract (0.5 mg/vial)
VII	Fructose (5 mg/vial) + PC Methanol Extract (2.0 mg/vial)

^aThe order of the samples was random to eliminate bias during scoring.

The results from this experiment are found on the attached data sheets which indicate insect mortality (+ = alive, - = dead). Twenty larvae were tested per sample. They were scored for mortality after two,⁵ five,⁶ eight⁷ and twelve days.⁸ Observations made after eight days gave definitive results while the controls with no food were still viable. From the data the following conclusions were made:

1. CB larvae can survive without a food source for enough time to observe sample toxicity.
2. Fructose provides a good nutrient supply for CB larvae.
3. The PC methanol extract is toxic to CB larvae.
4. The design of this experiment is extremely useful and provides a faster and simpler means of bioassay.

Further experiments are in progress to determine if a dose response to the PC methanol extract can be established and to ascertain if the larvicidal activity extends to other insects.

References

1. Tafur, S. P.M. Notebook No. 7778, p. 120-121.
2. Faustini, D. P.M. Notebook No. 7746, p. 103-104.
3. Faustini, D. P.M. Notebook No. 7746, p. 82.

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4. Tafur, S. P.M. Notebook No. 7778, p. 113.
5. Zimmermann, M. P.M. Notebook No. 7910, p. 66.
6. Kinser, R. P.M. Notebook No. 7939, p. 2.
7. Faustini, D. P.M. Notebook No. 7746, p. 104.
8. Zimmermann, M. P.M. Notebook No. 7910, p. 67.

ps

Attachments

cc: T. S. Osdene ✓
S. A. Hutcheson
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DATE 18 July 83

SET-UP 01
JULY 06, 1983

+ = ALIVE
- = DEAD

? = looks alive, not described nor relayed
but doesn't move

I II III IV V VI VII

1	+	-	+	+	?	+	?
2	+	-	-	+	?	+	+
3	+	-	-	+	2	+	?
4	+	-	+	+	2	+	+
5	+	-	+	+	?	-	?
6	+	-	-	+	2	+	?
7	+	-	+	+	+	+	?
8	+	-	+	+	?	+	?
9	+	-	-	+	?	-	+
10	+	-	+	+	2	+	2
11	+	-	-	+	?	+	2
12	+	-	+	+	+	-	2
13	+	-	+	+	?	+	2
14	+	-	-	+	2	+	-
15	+	-	-	+	2	+	?
16	+	-	+	+	?	+	?
17	+	-	+	+	?	-	?
18	+	-	-	+	?	+	?
19	+	-	+	+	?	-	?
20	+	-	+	+	+	+	?

0/0 20/25 8/20 0/20
all alive
to want to
know who
all
dead
all
alive
all
dead

all alive
to want to
know who
all
dead
all
alive
all
dead

OBSERVATION DATES

JULY 08th, 11th, 14th, 18th, 22th

white
brown
pink
1:3

2001214535

DATE: 11 July 83

SET-UP ON
JULY 06, 1983.

+ = ALIVE
- = DEAD

	I	II	III	IV	V	VI	VII
1	+	-	-	+	-	-	-
2	+	-	-	+	-	-	+
3	+	-	-	+	?	+	?
4	+	-	-	+	-	-	-
5	+	-	-	+	-	-	?
6	+	-	-	+	-	-	+
7	+	-	-	+	-	-	?
8	+	-	-	+	-	-	?
9	+	-	-	+	-	-	?
10	+	-	-	+	-	-	-
11	+	-	-	+	-	-	-
12	+	-	-	+	-	-	+
13	+	-	-	+	-	-	?
14	+	-	-	+	-	-	?
15	+	-	-	+	-	-	-
16	+	-	-	+	-	-	-
17	+	-	-	+	-	-	?
18	+	-	-	+	-	-	?
19	+	-	-	+	-	-	-
20	+	-	-	+	-	-	-

OBSERVATION DATES: 11th, 14th, 18th, 21st
JULY 08: 11th, 14th, 18th, 21st

I-20: Bird whisk + pump, but no movement

VI-11
VI-13
VI-16

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DATE

July 14, 1983

SET-UP ON
JULY 06, 1983+ = ALIVE
- = DEAD

	I	II	III	IV	V	VI	VII
1	+	-	-	+	-	-	-
2	+	-	-	+	-	-	+
3	+	-	-	+	-	-	-
4	+	-	-	+	-	-	-
5	+	-	-	+	-	-	-
6	-	-	-	+	-	-	-
7	+	-	-	+	+	-	-
8	+	-	-	+	-	-	-
9	+	-	-	-	-	-	-
10	+	-	-	+	-	-	-
11	+	-	-	+	-	-	-
12	+	-	-	+	-	-	-
13	+	-	-	+	-	-	-
14	-	-	-	+	-	-	-
15	+	-	-	+	-	-	-
16	+	-	-	+	-	-	-
17	-	-	-	+	-	-	-
18	+	-	-	+	-	-	-
19	+	-	-	+	-	-	-
20	+	-	-	+	-	-	-

3/20 20/20 20/20 1/20 19/20 20/20 19/20

OBSERVATION DATES +1

JULY 08, 11, 14, 18, 21

dead / total

2001214537

2001214539

T. S. OSDENE

AUG 8 1983